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☐ **1:** Corti O, Sanchez-Capelo A, Colin P, Hanoun N, Hamon M, Mallet J. Related Articles, Lin

Long-term doxycycline-controlled expression of human tyrosine hydroxylase after direct adenovirus-mediated gene transfer to a rat model of Parkinson's disease.
Proc Natl Acad Sci U S A. 1999 Oct 12;96(21):12120-5.
PMID: 10518586 [PubMed - indexed for MEDLINE]

☐ **2:** Sanchez-Capelo A, Corti O, Mallet J. Related Articles, Lin

Adenovirus-mediated over-expression of TGFbeta1 in the striatum decreases dopaminergic cell survival in embryonic nigral grafts.
Neuroreport. 1999 Jul 13;10(10):2169-73.
PMID: 10424693 [PubMed - indexed for MEDLINE]

☐ **3:** Corti O, Sabate O, Horellou P, Colin P, Dumas S, Buchet D, Buc-Caron MH, Mallet J. Related Articles, Lin

A single adenovirus vector mediates doxycycline-controlled expression of tyrosine hydroxylase in brain grafts of human neural progenitors.
Nat Biotechnol. 1999 Apr;17(4):349-54.
PMID: 10207882 [PubMed - indexed for MEDLINE]

☐ **4:** Ridet JL, Corti O, Pencalet P, Hanoun N, Hamon M, Philippon J, Mallet J. Related Articles, Lin

Toward autologous ex vivo gene therapy for the central nervous system with human adult astrocytes.
Hum Gene Ther. 1999 Jan 20;10(2):271-80.
PMID: 10022551 [PubMed - indexed for MEDLINE]

☐ **5:** Barkats M, Bilang-Bleuel A, Buc-Caron MH, Castel-Barthe MN, Corti O, Finiels F, Horellou P, Revah F, Sabate O, Mallet J. Related Articles, Lin

Adenovirus in the brain: recent advances of gene therapy for neurodegenerative diseases.
Prog Neurobiol. 1998 Jul;55(4):333-41. Review.
PMID: 9654383 [PubMed - indexed for MEDLINE]

☐ **6:** Corti O, Horellou P, Colin P, Cattaneo E, Mallet J. Related Articles, Lin

Intracerebral tetracycline-dependent regulation of gene expression in grafts o neural precursors.
Neuroreport. 1996 Jul 8;7(10):1655-9.
PMID: 8904776 [PubMed - indexed for MEDLINE]

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 FILE 'MEDLINE, EMBASE, BIOSIS' ENTERED AT 14:42:12 ON 13 DEC 2004
L1         12562 S MALLET?/AU OR CORTI?/AU
L2           346 S MCGEADY?/AU
L3             6 S L2 AND UMS
L4             2 DUP REM L3 (4 DUPLICATES REMOVED)
L5        208198 S HI
L6           622 S PGK (P) PROMOTER
L7             1 S TETRACYLINE (P) OPERATOR
L8           285 S "TTA" (S) TRANSACTIVATOR
L9           230 S UMS OR "UPSTREAM MOUSE SEQUENCE"
L10            3 S "PHTS3MS"
L11            1 DUP REM L10 (2 DUPLICATES REMOVED)
L12          176 S "TTA" AND "TET"
L13            0 S L12 AND L9
L14            0 S L8 AND L9
L15            0 S L6 AND L9
L16            4 S L6 AND L8
L17            3 DUP REM L16 (1 DUPLICATE REMOVED)
L18            0 S "TETRACYCLINEREGULATED SYSTEM"
L19          115 S "TETRACYCLINE REGULATED SYSTEM"
L20            0 S L19 AND L9
L21          101 S L19 AND EXPRESSION
L22           19 S L21 AND VECTOR
L23           12 DUP REM L22 (7 DUPLICATES REMOVED)
L24            2 S L23 NOT PY>=2000
L25            0 S L1 AND L19
L26            0 S L1 AND L9
L27            0 S L1 AND L8
L28            0 S L1 AND L6
L29            0 S L6 AND L19
L30         3656 S CMV (P) PROMOTER
L31            0 S L6 AND L30 AND (L19 OR L12)
L32           54 S L6 AND L30
L33            0 S L32 AND L19
L34            1 S L32 AND TET
L35          439 S BUJARD?/AU
L36            1 S L35 AND L19
L37       244276 S HIS
L38         1653 S TET (P) (OPERON OR PROMOTER OR "ON SYSTEM" OR ACTIVATOR OR RE
L39            2 S L38 AND L6
L40            2 DUP REM L39 (0 DUPLICATES REMOVED)
L41            6 S L38 AND "EXPRESSION CONSTRUCT"
L42            2 DUP REM L41 (4 DUPLICATES REMOVED)
L43            0 S L38 AND L10
L44           11 S L38 AND L19
L45            7 DUP REM L44 (4 DUPLICATES REMOVED)
L46            3 S L45 NOT PY>=2001

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L2	135467	promoter or terminator	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/12/13 16:19
L3	3432	((tetracycline and tTA) or UMS or hTH-1 or ppgk or PIX) and (promoter or terminator)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/12/13 16:19
L4	39328	(promoter or terminator) SAME (tissue specific)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/12/13 16:19
L5	2099	((tetracycline and tTA) or UMS or hTH-1 or ppgk or PIX) and ((promoter or terminator) SAME (tissue specific))	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/12/13 16:19
L6	0	adpgk WITH tet	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/12/13 16:19
L7	90	"protein IX" SAME adenoviral	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/12/13 16:19
L8	57	("protein IX" SAME adenoviral) and ((tetracycline and tTA) or UMS or hTH-1 or ppgk or PIX)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/12/13 16:19
L9	2319	Tn10 or "tetracycline operon"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/12/13 16:19
L10	6	adenovrial and ("gene regulation" or "gene activity" or "gene expression")	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/12/13 16:19
L11	30305	adenovirus	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/12/13 16:19

L12	25393	gene WITH (express? or regulat? or activ?)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/12/13 16:19
L13	270	(Tn10 or "tetracycline operon") and adenovirus and (gene WITH (express? or regulat? or activ?))	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/12/13 16:19
L14	269	((Tn10 or "tetracycline operon") and adenovirus and (gene WITH (express? or regulat? or activ?))) and (promoter or terminator)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/12/13 16:19
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L16	0	tetracyline WITH "responsive regulatory system"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/12/13 16:19
L17	0	tetracyline SAME "responsive regulatory system"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/12/13 16:19
L18	540	tet-off or "tet off"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/12/13 16:19
L19	2	((((Tn10 or "tetracycline operon") and adenovirus and (gene WITH (express? or regulat? or activ?))) and (promoter or terminator)) and ((tetracycline and tTA) or UMS or hTH-1 or ppgk or PIX)) and (tet-off or "tet off")	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/12/13 16:19
L20	9	reeves.in. and "retroviral"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/12/13 16:19
L21	3680	tyrosine and hydroxylase	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/12/13 16:19
L22	17157	cmv and promoter	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/12/13 16:19

L23	44485	tet or tetracycline or "tet operon" or operon	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/12/13 16:19
L24	918	(tyrosine and hydroxylase) and (cmv and promoter) and (tet or tetracycline or "tet operon" or operon)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/12/13 16:19
L25	0	((tyrosine and hydroxylase) and (cmv and promoter) and (tet or tetracycline or "tet operon" or operon)) and adenovir?	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/12/13 16:19
L26	131	((tyrosine and hydroxylase) and (cmv and promoter) and (tet or tetracycline or "tet operon" or operon)) and pgk	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/12/13 16:19
L27	3	"upstream mouse sequence"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/12/13 16:19
L28	8	"6552003".pn. or "6432701".pn. or "6632427".pn. or "6756523".pn.	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/12/13 16:19
L29	16611	PGK or EF1 or beta-actin or vimentin or adolase or antitrypsin	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/12/13 16:19
L30	1208	(PGK or EF1 or beta-actin or vimentin or adolase or antitrypsin) and (tyrosine and hydroxylase)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/12/13 16:19
L31	700	((PGK or EF1 or beta-actin or vimentin or adolase or antitrypsin) and (tyrosine and hydroxylase)) and (tet or tetracycline or "tet operon" or operon)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/12/13 16:19
L32	550	(((PGK or EF1 or beta-actin or vimentin or adolase or antitrypsin) and (tyrosine and hydroxylase)) and (tet or tetracycline or "tet operon" or operon)) and (cmv and promoter)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/12/13 16:19
L33	149	((((PGK or EF1 or beta-actin or vimentin or adolase or antitrypsin) and (tyrosine and hydroxylase)) and (tet or tetracycline or "tet operon" or operon)) and (cmv and promoter)) and (terminator or silenc?)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/12/13 16:19

L34	8188939	"WO" (s) "20463"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/12/13 16:23
L35	2	"WO 97/20463"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/12/13 16:24
L36	1	"WO 98/37185"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/12/13 16:27
L37	0	"WO98/37185"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/12/13 16:27
L38	0	"PCT/US98/03092"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/12/13 16:28
L39	0	"US98/03092"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/12/13 16:28
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L42	2	"9720463"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/12/13 16:29
L43	0	l41 and (nonviral or non-viral)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/12/13 16:52
L44	7142170	(cell-specific or tissue-specific) (s) promoter	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/12/13 16:53
L45	2	l41 and l44	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/12/13 16:53

L46	32768	"cell specific" or "tissue specific"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/12/13 16:54
L47	0	l46 and l41	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/12/13 16:54
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S2	126027	promoter or terminator	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/06/23 18:25
S3	3076	((tetracycline and tTA) or UMS or hTH-1 or ppgk or PIX) and (promoter or terminator)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/06/23 18:26
S4	35588	(promoter or terminator) SAME (tissue specific)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/06/23 18:26
S5	1851	((tetracycline and tTA) or UMS or hTH-1 or ppgk or PIX) and ((promoter or terminator) SAME (tissue specific))	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/06/23 18:27
S6	0	adpgk WITH tet	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/06/23 18:27
S7	85	"protein IX" SAME adenoviral	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/06/23 18:28
S8	55	("protein IX" SAME adenoviral) and ((tetracycline and tTA) or UMS or hTH-1 or ppgk or PIX)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/06/23 18:30
S9	2057	Tn10 or "tetracycline operon"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/06/23 18:30
S10	4	adenovrial and ("gene regulation" or "gene activity" or "gene expression")	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/06/23 18:31

S11	27417	adenovirus	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/06/23 18:31
S12	22990	gene WITH (express? or regulat? or activ?)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/06/23 18:32
S13	240	(Tn10 or "tetracycline operon") and adenovirus and (gene WITH (express? or regulat? or activ?))	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/06/23 18:33
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S16	0	tetracyline WITH "responsive regulatory system"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/06/23 18:35
S17	0	tetracyline SAME "responsive regulatory system"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/06/23 18:35
S18	460	tet-off or "tet off"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/06/23 18:36
S19	2	((((Tn10 or "tetracycline operon") and adenovirus and (gene WITH (express? or regulat? or activ?))) and (promoter or terminator)) and ((tetracycline and tTA) or UMS or hTH-1 or ppgk or PIX)) and (tet-off or "tet off")	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/06/23 18:36
S20	8	reeves.in. and "retroviral"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/06/24 11:44
S21	3287	tyrosine and hydroxylase	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/06/24 11:44

S22	15263	cmv and promoter	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/06/24 11:44
S23	40965	tet or tetracycline or "tet operon" or operon	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/06/24 11:45
S24	826	(tyrosine and hydroxylase) and (cmv and promoter) and (tet or tetracycline or "tet operon" or operon)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/06/24 11:45
S25	0	((tyrosine and hydroxylase) and (cmv and promoter) and (tet or tetracycline or "tet operon" or operon)) and adenovir?	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/06/24 11:45
S26	116	((tyrosine and hydroxylase) and (cmv and promoter) and (tet or tetracycline or "tet operon" or operon)) and pgk	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/06/24 11:55
S27	3	"upstream mouse sequence"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/06/24 12:03
S28	6	"6552003".pn. or "6432701".pn. or "6632427".pn. or "6756523". pn.	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/06/24 12:22
S29	15077	PGK or EF1 or beta-actin or vimentin or adolase or antitrypsin	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/06/24 12:23
S30	1078	(PGK or EF1 or beta-actin or vimentin or adolase or antitrypsin) and (tyrosine and hydroxylase)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/06/24 12:23
S31	629	((PGK or EF1 or beta-actin or vimentin or adolase or antitrypsin) and (tyrosine and hydroxylase)) and (tet or tetracycline or "tet operon" or operon)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/06/24 12:23
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S33	135	((((PGK or EF1 or beta-actin or vimentin or adolase or antitrypsin) and (tyrosine and hydroxylase)) and (tet or tetracycline or "tet operon" or operon)) and (cmv and promoter)) and (terminator or silenc?)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2004/06/24 12:24

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ACCESSION NUMBER: 2004:197101 BIOSIS
DOCUMENT NUMBER: PREV200400197660
TITLE: Towards conditional lentivector - mediated GDNF expression in vivo.
AUTHOR(S): Szulc, J. [Reprint Author]; Spicher, A. [Reprint Author]; Deglon, N. [Reprint Author]; Aebischer, P. [Reprint Author]
CORPORATE SOURCE: Inst. of Neurosci., Swiss Federal Inst. of Technol., Lausanne, Switzerland
SOURCE: Society for Neuroscience Abstract Viewer and Itinerary Planner, (2003) Vol. 2003, pp. Abstract No. 299.9. http://sfn.scholarone.com. e-file.
Meeting Info.: 33rd Annual Meeting of the Society of Neuroscience. New Orleans, LA, USA. November 08-12, 2003. Society of Neuroscience.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 14 Apr 2004
Last Updated on STN: 14 Apr 2004

AB Lentiviral expression of glial cell-line derived neurotrophic factor (GDNF) in the striatum was shown to prevent neurodegeneration and promote the sprouting of remaining dopaminegic fibers in both rat and primate models of Parkinson's disease (PD). Since continuous GDNF expression may cause serious side effects we used tetracycline-inducible system (TET) to control its expression. Two vectors, one carrying GDNF under control of inducible tetO **promoter** and the other encoding for tetracycline **transactivator** (**tTA**) were unilaterally injected into rat striata, followed by doxycycline (dox) administration. A 100-fold induction of GDNF expression was observed in a group that did not receive dox as compared to intact animals. However, significant, non-specific transgene expression was observed in striata of dox treated animals. In order to overcome this limitation, tTA was exchanged for a tetracycline transrepressor (tTR-KRAB). While, tight transgene repression was observed in the absence of dox in the group of rats intrastriatally injected with two vectors, tetO-mediated transcription in the presence of dox yielded only low GDNF expression. Consequently, we developed a strategy allowing conditional repression of strong murine **PGK promoter** via a dox-controllable tTR-KRAB binding to tetO. Importantly, by expressing GDNF as a part of bicistronic unit together with tTR-KRAB and inserting tetO sequences into LTRs, we incorporated the TET/repressor system into a single vector. The major advantage of single vector design is regulation of transgene expression in every transduced cell in vivo. Transduction of cell lines with constructed lentivector resulted in tight and efficient regulation of GFP marker and GDNF protein. GDNF expression is presently tested in vivo. Due to its simplicity and efficacy, single vector design holds the most promise and may facilitate clinical application of GDNF-based gene therapy for PD.

L17 ANSWER 2 OF 3 MEDLINE on STN
ACCESSION NUMBER: 2003317500 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12392602
TITLE: Retroviral vectors for establishing tetracycline-regulated gene expression in an otherwise recalcitrant cell line.
AUTHOR: Kenny Paraic A; Enver Tariq; Ashworth Alan
CORPORATE SOURCE: Section of Gene Function and Regulation, Institute of Cancer Research, Chester Beatty Laboratories, 237 Fulham Road, London SW3 6JB, United Kingdom.. pakenny@lbl.gov
SOURCE: BMC molecular biology [electronic resource], (2002 Sep 3) 3 (1) 13.
Journal code: 100966983.
PUB. COUNTRY: England: United Kingdom

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: PUBMED-NOT-MEDLINE
ENTRY MONTH: 200310
ENTRY DATE: Entered STN: 20030709
Last Updated on STN: 20031101
Entered Medline: 20031031

AB BACKGROUND: Tetracycline-regulated systems have been used to control the expression of heterologous genes in such diverse organisms as yeast, plants, flies and mice. Adaptation of this prokaryotic regulatory system avoids many of the problems inherent in other inducible systems. There have, however, been many reports of difficulties in establishing functioning stable cell lines due to the cytotoxic effects of expressing high levels of the tetracycline **transactivator, tTA,** from a strong viral **promoter.** RESULTS: Here we report the successful incorporation of tetracycline-mediated gene expression in a mouse mammary epithelial cell line, HC11, in which conventional approaches failed. We generated retroviruses in which tTA expression was controlled by one of three promoters: a synthetic tetracycline responsive **promoter** (TRE), the elongation factor 1-alpha **promoter** (EF1alpha) or the phosphoglycerate kinase-1 **promoter** (**PGK**), and compared the resulting cell lines to one generated using a cytomegalovirus immediate early gene **promoter** (CMV). In contrast to cells produced using the CMV and **PGK** promoters, those produced using the EF1alpha and TRE promoters expressed high levels of beta-galactosidase in a tetracycline-dependent manner. CONCLUSIONS: These novel retroviral vectors performed better than the commercially available system and may have a more general utility in similarly recalcitrant cell lines.

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ACCESSION NUMBER: 2004236317 EMBASE
TITLE: Retroviral vectors for establishing tetracycline-regulated gene expression in an otherwise recalcitrant cell line.
AUTHOR: Kenny P.A.; Enver T.; Ashworth A.
CORPORATE SOURCE: P.A. Kenny, Life Sciences Division, Lawrence Berkeley Natl. Laboratory, 1 Cyclotron Road, Berkeley, CA 94720, United Kingdom. pakenny@lbl.gov
SOURCE: BMC Molecular Biology, (3 Sep 2002) 3/-.
Refs: 31
ISSN: 1471-2199 CODEN: BMBMC4
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Background: Tetracycline-regulated systems have been used to control the expression of heterologous genes in such diverse organisms as yeast, plants, flies and mice. Adaptation of this prokaryotic regulatory system avoids many of the problems inherent in other inducible systems. There have, however, been many reports of difficulties in establishing functioning stable cell lines due to the cytotoxic effects of expressing high levels of the tetracycline **transactivator, tTA,** from a strong viral **promoter.** Results: Here we report the successful incorporation of tetracycline-mediated gene expression in a mouse mammary epithelial cell line, HC11, in which conventional approaches failed. We generated retroviruses in which **tTA** expression was controlled by one of three promoters: a synthetic tetracycline responsive **promoter** (TRE), the elongation factor 1-alpha **promoter** (EF1α) or the phosphoglycerate kinase-1 **promoter** (**PGK**), and compared the resulting cell lines to one generated using

a cytomegalovirus immediate early gene **promoter** (CMV). In contrast to cells produced using the CMV and **PGK** promoters, those produced using the EF1α and TRE promoters expressed high levels of β-galactosidase in a tetracycline-dependent manner. Conclusions: These novel retroviral vectors performed better than the commercially available system and may have a more general utility in similarly recalcitrant cell lines.

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E: DNA (Mary Ann Liebert, Inc.), (1986 Aug) 5 (4) 289-98.
Journal code: 8302432. ISSN: 0198-0238.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-M13896
ENTRY MONTH: 198610
ENTRY DATE: Entered STN: 19900321
Last Updated on STN: 19970203
Entered Medline: 19861022

AB A region upstream from the mouse c-mos proto-oncogene, termed upstream mouse sequence (**UMS**), prevents expression of mos transforming activity. Previous studies suggested that the **UMS** prevented transcription readthrough. In this study, we constructed a recombinant DNA clone, pHTS3MS, with the **UMS** inserted downstream from both the mos gene and a truncated long terminal repeat containing only the U3 enhancer region. In this position **UMS** did not inhibit mos transforming activity. We examined cells transformed by pHTS3MS for RNA expression. S1 nuclease analysis showed that the **UMS** provides two polyadenylation signals to mos-containing RNA and nuclear run-on transcription showed that the primary transcripts terminate in **UMS** . In addition, using portions of the **UMS**, we found that a 360-bp fragment containing the **UMS** polyadenylation signals and sites inserted between the herpes simplex virus type 1 (HSV-1) thymidine kinase gene (tk) and its promoter inhibits tk transforming activity by 99% and prevents detectable expression of this construct in transient expression assays. Thus, the **UMS** must contain signals for polyadenylation and appears to function as a transcription terminator.

L4 ANSWER 2 OF 2 MEDLINE on STN DUPLICATE 2
ACCESSION NUMBER: 85088498 MEDLINE
DOCUMENT NUMBER: PubMed ID: 6096859
TITLE: Mouse c-mos oncogene activation is prevented by upstream sequences.
AUTHOR: Wood T G; **McGeady M L**; Baroudy B M; Blair D G; Vande Woude G F
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (1984 Dec) 81 (24) 7817-21.
Journal code: 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-J00371; GENBANK-J00372
ENTRY MONTH: 198502
ENTRY DATE: Entered STN: 19900320
Last Updated on STN: 19970203
Entered Medline: 19850221

AB Although the molecularly cloned mouse c-mos oncogene locus can be efficiently activated by insertion of a retroviral long terminal repeat (LTR) 5' to its coding region, only low-frequency transformation occurs with the LTR element inserted 3' to this region. Analysis of several of the latter transformed cell lines suggested that loss of 2 kilobases (kb) of normal mouse DNA sequences preceding c-mos was required for oncogene activation. The determination of the transforming potential of deletion mutants containing only portions of this region followed by analysis of their nucleotide sequences identified a region termed upstream mouse sequence (**UMS**) as a cis-acting locus that prevents c-mos activation by a 3' LTR. The **UMS** region is approximately 1 kb in length and is located 0.8-1.8 kb upstream from the first ATG in the open reading frame of c-mos. Insertion of **UMS** 5' to the v-mos coding

region also prevents 3' LTR enhancement of its transforming activity, but this inhibition is position dependent and functions only when inserted between v-mos and its putative promoter. The results presented here suggest that **UMS** may function to regulate c-mos proto-oncogene expression and may explain the lack of detectable c-mos transcripts in normal mouse cells.

L46 ANSWER 1 OF 3 MEDLINE on STN
ACCESSION NUMBER: 1999221888 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10203578
TITLE: Expression of green fluorescent protein in oligodendrocytes in a time- and level-controllable fashion with a **tetracycline-regulated system**.
AUTHOR: Huang C J; Spinella F; Nazarian R; Lee M M; Dopp J M; de Vellis J
CORPORATE SOURCE: Departments of Neurobiology and Psychiatry, Brain Research Institute, Mental Retardation Research Center, UCLA School of Medicine, Los Angeles, California 90024, USA.
CONTRACT NUMBER: HD 06576 (NICHD)
HD 07032 (NICHD)
SOURCE: Molecular medicine (Cambridge, Mass.), ~~(1999 Feb) 5~~; (2) 129-37.
Journal code: 9501023. ISSN: 1076-1551.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199906
ENTRY DATE: Entered STN: 19990628
Last Updated on STN: 19990628
Entered Medline: 19990611

AB Developments in transgenic technology have greatly enhanced our ability to understand the functions of various genes in animal models and relevant human diseases. The tetracycline (**tet**)-**regulated** transactivation system for inducing gene expression allowed us to control the expression of exogenous genes in a temporal and quantitative way. The ability to manipulate a cell-specific **promoter** enabled us to express one particular protein in a single type of cell. The combination of a tetracycline system and a tissue-specific **promoter** has led us to the development of an innovative gene expression system, which is able to express genes in a cell type-specific and time- and level-controllable fashion. An oligodendrocyte-specific myelin basic protein (MBP) gene **promoter** controls the reversed **tet** -inducible transactivator. The green fluorescent protein (GFP) gene was placed under the control of the human cytomegalovirus (CMV) basic **promoter** in tandem with seven **tet**-responsive elements (TRE), binding sites for the activated transactivator. Upon the addition of doxycycline (DOX, a tetracycline derivative), **tet** transactivators became activated and bound to one or more TRE, leading to the activation of the CMV **promoter** and the expression of GFP in oligodendrocytes. We have successfully expressed GFP and luciferase at high levels in oligodendrocytes in a time- and dose-dependent fashion. In the absence of DOX, there was almost no GFP expression in oligodendroglial cultures. Graded levels of GFP expression were observed after induction with DOX (0.5 to 12.5 microg/ml). Our data indicate that this inducible gene expression system is useful for the study of gene function in vivo and for the development of transgenic animal models relevant to human diseases such as multiple sclerosis.

L46 ANSWER 2 OF 3 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN
ACCESSION NUMBER: 2001:108518 BIOSIS
DOCUMENT NUMBER: PREV200100108518
TITLE: Multiple lines of mice with inducible region-specific expression of high affinity nicotinic receptors.
AUTHOR(S): King, S. L. [Reprint author]; Kelz, M. B.; Steffen, C.; Chen, J.; Koren, A. O.; Mukhin, A. G.; Nestler, E. J.; Picciotto, M. R.
CORPORATE SOURCE: Yale Univ. Sch. of Med., New Haven, CT, USA

SOURCE: Society for Neuroscience Abstracts, (2000) Vol. 26, No. 1-2, pp. Abstract No.-565.14. print.
Meeting Info.: 30th Annual Meeting of the Society of Neuroscience. New Orleans, LA, USA. November 04-09, 2000. Society for Neuroscience.
ISSN: 0190-5295.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 28 Feb 2001
Last Updated on STN: 15 Feb 2002

AB Mice lacking the beta2 subunit of the nicotinic acetylcholine receptor (nAChR) lack high affinity nicotine binding sites and show behavioral differences compared to their wild type siblings in learning and reinforcement paradigms. Using a **tetracycline regulated system** we have generated mice expressing the beta2 subunit in the brain in a regionally and temporally specific manner. Crossing different **tet**-transactivator lines with tetracycline **regulated** beta2 lines and beta2 knock out mice results in distinct patterns of nAChR expression in the brain. We have characterized multiple lines of mice with different patterns of nAChR expression using equilibrium binding with radio-iodinated analogs of the nicotinic agonists epibatidine and A85380. We have generated mouse lines that express the beta2 subunit predominantly in the thalamus and cortex, with some expression in the hippocampus, as well as lines with expression restricted to a small subset of thalamic and mammillary nuclei. Analysis of other lines is in progress. Expression of these receptors can also be **regulated** temporally. Expression was eliminated by treating the animals with doxycycline. Preliminary experiments showed that restoration of beta2 subunit expression in the thalamus and cortex rescued the baseline change in passive avoidance behavior seen in knock out mice. Expressing the beta2 subunit of the nAChR in a restricted manner will allow us to pinpoint the anatomical sites for nicotine's actions in different behaviors.

L46 ANSWER 3 OF 3 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN
ACCESSION NUMBER: 1999:203959 BIOSIS
DOCUMENT NUMBER: PREV199900203959
TITLE: In vivo manipulation of interleukin-2 expression by a retroviral tetracycline (**tet**)-**regulated** system.
AUTHOR(S): Pitzer, Claudia; Schindowski, Katharina; Pomer, Sigmund; Wirth, Thomas; Zoeller, Margot [Reprint author]
CORPORATE SOURCE: Department of Tumor Progression and Immune Defense, German Cancer Research Center, Im Neuenheimer Feld 280, D-69120, Heidelberg, Germany
SOURCE: Cancer Gene Therapy, (March-April, 1999) Vol. 6, No. 2, pp. 139-146. print.
ISSN: 0929-1903.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 26 May 1999
Last Updated on STN: 26 May 1999

AB We have used the tetracycline (**tet**)-**regulated** system as described previously to evaluate the applicability of controlled gene expression in cancer gene therapy. As a model gene, we used the human interleukin-2 (IL-2) gene, which has been placed under the transcriptional control of the tetO/**promoter**. Human melanoma cells were transduced by two modified retroviral **tet** vectors containing the transactivator regulatory unit and the IL-2 gene driven by the tetO/**promoter**, respectively. In the absence of **tet**, IL-2 expression in the target cells was stable over several months. IL-2 production was in the range of 40 U/106 cells/24 hours. A fine tuning of

IL-2 expression could be achieved by culturing the transduced cells with increasing doses of **tet**, whereby a concentration of 500 ng/mL **tet** in the culture medium abrogated IL-2 expression. Most importantly for clinical application, IL-2 expression by the transduced melanoma cells could also be **regulated** in vivo. When nu/nu mice were inoculated with the transduced tumor cells, they failed to develop tumors. Instead, the inhibition of IL-2 expression in the transduced tumor cells by oral administration of **tet** led to subcutaneous tumor growth; this growth rate was comparable with the growth rate of subcutaneously inoculated untransduced parental cells. The finding demonstrates the applicability of the **tet-regulated** system in cancer gene therapy.

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